Protease Inhibitors in Spontaneous Cervical Artery Dissections

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Background and Purpose—Observations in patients with arterial aneurysms, fibromuscular dysplasia, and spontaneous cervical artery dissection (sCAD) indicate that protease inhibitor deficiency might boost the enzymatic destruction of arterial tissue and increase the risk of these arterial wall diseases. Here we present the first large investigation of the protease inhibitor hypothesis in patients with sCAD.

Methods—Eighty patients with sCAD were compared with 80 age- and sex-matched healthy individuals. \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-AT) and \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)-MG) levels, and \( \alpha_1 \)-AT genotypes were assessed and compared between groups.

Results—\( \alpha_1 \)-AT and \( \alpha_2 \)-MG levels as well as \( \alpha_1 \)-AT genotypes did not differ significantly between patients and controls. The frequency of Z alleles in the patient group was higher than in the control group and than in other cohorts from Europe; however, the difference remained nonsignificant. All patients with Z alleles had internal carotid artery dissections.

Conclusions—Overall, this data does not support the hypothesis that protease inhibitor levels or \( \alpha_1 \)-AT genotypes play an important role in the etiology of sCAD. The present data does not exclude that the \( Pi-Z \) allele might have an influence on subgroups of sCAD, such as internal carotid artery dissections. (Stroke. 2005;36:9-13.)

Key Words: alpha 1-antitrypsin ■ alpha-macroglobulins ■ dissection ■ protease inhibitors ■ risk factors

With an estimated annual incidence of \( \approx 2.6 \) per 100 000, spontaneous cervical artery dissection (sCAD) is a rare disease in neurological practice.1 Nevertheless, it is an important cause of stroke: 13% to 15.5% of strokes in adults <45 years2,3 and 30% to 40% of brain stem and cerebellar infarctions in this population occur on account of sCAD.4,5 Pathophysiologically, rupture of either the arterial intimal layer or the medial/adventitial layer, including vasa vasorum, causes an intramural arterial hematoma often leading to stenosis with a high risk of embolic brain infarction.6,7 Association of sCAD with heritable connective tissue diseases, such as Ehlers-Danlos syndrome type IV and Marfan syndrome,8,9 and ultrastructural connective tissue abnormalities in skin biopsies of sCAD patients6,10 suggest an important etiologic role of connective tissue aberrations in sCAD.

Recently, a number of case reports on patients with protease inhibitor deficiency and sCAD have been published, suggesting a possible etiologic role of protease inhibitor deficiency for sCAD.11–13 In a small series of 22 patients with sCAD, 27.3% showed low levels of \( \alpha_1 \)-antitrypsin (\( \alpha_1 \)-AT),14 whereas no effect of \( \alpha_1 \)-AT was observed in 35 patients with sCAD.15 Just recently, an investigation of \( \alpha_1 \)-AT deficiency alleles in 74 patients did not suggest a causal relationship between \( \alpha_1 \)-AT alleles and sCAD.16 For other arterial wall pathologies, an association of reduced antiproteolytic activity has repeatedly been observed, including patients with arterial aneurysms17–19 or fibromuscular dysplasia.20–22 On the basis of these observations, it has been postulated that an imbalance of proteolytic and antiproteolytic enzymatic activity might be a possible risk factor for sCAD.11–13 However, larger studies on proteases inhibitor levels and genotypes in patients with sCAD are missing so far.

The major inhibitors of human proteinases are \( \alpha_1 \)-AT and \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)-MG). The 2 most important genetic variants leading \( \alpha_1 \)-AT deficiency are named \( S \) and \( Z \) alleles. The normal variant is called \( M \) allele. \( S \) and \( Z \) alleles are caused by point mutations leading to amino acid exchanges (\( S \) allele: glutamic acid 264 to valine; \( Z \) allele: glutamic acid 342 to lysine). Severe \( \alpha_1 \)-AT deficiency is in the majority of cases associated with the \( Pi-Z \) genotype and causes severe damage of connective tissues in lungs, liver, and skin.23 In this study, we tested the hypothesis that reduced antiproteolytic enzyme activity may increase the risk of cervical arterial dissections by assessing \( \alpha_1 \)-AT serum levels beyond the acute phase, \( \alpha_1 \)-AT genotype, and \( \alpha_2 \)-MG serum levels in a large group of patients with sCAD and age- and sex-matched healthy controls population.24–30

Received April 22, 2004; final revision received August 10, 2004; accepted October 6, 2004.
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Stroke is available at http://www.strokeaha.org
DOI: 10.1161/01.STR.0000149631.52985.27
Methods

Patient Population
All patients with the diagnosis of sCAD established in our department between 1992 and 2002 were contacted. The diagnosis of sCAD was based on clinical signs, that is either a local compressive syndrome or cerebral ischemia, and at least 1 sign confirming neuroradiological investigation (MRI with transversal sections through the neck or/and arterial digital subtraction angiography). Eighty patients, who gave written informed consent and had blood samples taken >1 month after sCAD to avoid the acute phase reaction, were included in the study. Excepting this, there were no other criteria for patients inclusion. An equal number of sex- and age-matched control subjects was drawn from the Prospective Cardiovascular Munster (PROCAM) study, a prospective population-based study on cardiovascular risk factors. EDTA-anticoagulated blood samples for genetic analysis could be obtained from 75 patients and 73 controls.

Biochemical and Genetic Analysis
For biochemical and genetic analysis, venous blood samples were taken in the early morning after overnight fasting (12 hours). The parameters α1-AT and α2-MG were determined immunonephelometrically with the Dade Behring BNII system using polyclonal antiserum from rabbit against these 2 proteins. C-reactive protein (CRP) was measured with the Roche Hitachi 747 system using Roche high-sensitive CRP reagents. For our laboratory, normal parameters were 90 to 200 μmol/L for α1-AT, 130 to 300 μmol/L for α2-MG, and <0.5 mg/dL for CRP. DNA was extracted from EDTA-anticoagulated blood samples with magnetic beads by Tecan DNA sample preparation system and frozen until analysis at −20°C. The α1-AT genotype was determined with the Light Cycler (Roche Diagnostics) according to a method from Aslandis et al11 using the following primers and probes from TIB MOLBIOL (Berlin, Germany): Pi*S allele: sense GGTGCTATATGATGAGGTTTAGGG; antisense AGGTGTGGGCACTTCTTGGTA; probe TCTCTCTGCTTGATGAGGAAACTA-fluorescein; reporter LC-red640-GACCAT-2-MG were determined immunonephelometrically with the Roche Hitachi 747 system using Roche high-sensitive CRP reagents. For our laboratory, normal levels were 90% below 1 mg/dL and 70% of CRP levels were 0.5 mg/dL in both groups and 0.235, P = 0.09, respectively). More than 70% of CRP levels were <0.5 mg/dL in both groups and >90% below 1 mg/dL. CRP did not significantly differ between patients and controls (Wilcoxon P = 0.129). Sixteen patients could remember a minor trauma before the symptoms of dissection, including working with hyperextension of the neck, rapid neck movements, etc. Fourteen patients had cervical chiropractic manipulation to alleviate neck pain before the diagnosis of sCAD. SCAD occurred in 4 vessels in 2 patients (both vertebral and both ICAs), 3 vessels in 1 patient (both vertebral and left ICA), 2 vessels in 14 patients (6 both vertebral arteries, 1 left vertebral and basilar artery, and 7 both ICAs), in 63 patients in 1 artery (22 vertebral and 41 ICAs). Fifty-seven patients experienced completed ischemic infarction, 23 had transient ischemic or local compressive symptoms. Median time between sCAD and blood withdrawal was 35.3 months (mean 40.7 ± 29.0).

α1-AT and α2-MG
Median α1-AT levels were 130.5 μmol/L in the patient group and 124.5 μmol/L in the control group (Wilcoxon P = 0.384). Median α2-MG levels were 159.5 μmol/L for patients and 146.5 μmol/L for controls (Wilcoxon P = 0.359). All α1-AT levels <90 μmol/L were associated with either Pi-S or Pi-Z alleles. α1-AT correlated with α2-MG and with CRP (Spearman correlation coefficient rs = 0.247, P = 0.004 and rs = 0.235, P = 0.006, respectively). The time between sCAD and blood withdrawal had no effect on α1-AT or log-transformed α2-MG levels (ANOVA P = 0.939 and P = 0.926, respectively). Gender had a significant influence on α1-AT and log-transformed α2-MG levels (ANOVA P = 0.007 and P = 0.001, respectively), with male subjects having lower median α1-AT and α2-MG levels than female subjects (124.0: 131.0 μmol/L and 142.0: 179.0 μmol/L, respectively). Age, smoking habits, diabetes, or hypertension had no influence on α1-AT and log-transformed α2-MG concentrations. α1-AT serum levels were significantly influenced by the α1-AT genotype: subjects with MM-genotype had a median α1-AT level of 127.5 μmol/L, MS had 118.0 μmol/L, MZ had 80.6 μmol/L, and ZZ had 20.9 μmol/L (ANOVA P = 0.001).

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients With sCAD</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>Gender (female: male)</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Smoker</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Minor trauma before sCAD</td>
</tr>
<tr>
<td>Chiropractic manipulation before sCAD</td>
</tr>
<tr>
<td>sCAD of carotid:vertebral:basilar artery</td>
</tr>
<tr>
<td>sCAD in 1:2:3:4 arteries</td>
</tr>
<tr>
<td>Completed strokes</td>
</tr>
</tbody>
</table>

Results
Thirty women and 50 men were included in each group, with a median age of 43.1 years (Table 1). χ² test with Fisher exact correction showed no significant differences between patients and controls concerning smoking, diabetes, and hypertension (P = 0.24, P = 1.00, and P = 0.09, respectively). More than 70% of CRP levels were <0.5 mg/dL in both groups and >90% below 1 mg/dL. CRP did not significantly differ between patients and controls (Wilcoxon P = 0.129). Sixteen patients could remember a minor trauma before the symptoms of dissection, including working with hyperextension of the neck, rapid neck movements, etc. Fourteen patients had cervical chiropractic manipulation to alleviate neck pain before the diagnosis of sCAD. SCAD occurred in 4 vessels in 2 patients (both vertebral and both ICAs), 3 vessels in 1 patient (both vertebral and left ICA), 2 vessels in 14 patients (6 both vertebral arteries, 1 left vertebral and basilar artery, and 7 both ICAs), in 63 patients in 1 artery (22 vertebral and 41 ICAs). Fifty-seven patients experienced completed ischemic infarction, 23 had transient ischemic or local compressive symptoms. Median time between sCAD and blood withdrawal was 35.3 months (mean 40.7 ± 29.0).
2). After adjustment for age and gender, there was still no significant difference between cases and controls. Introduction of CRP as a covariate does not change this result. Pathological $\alpha_1$-AT values occurred in 8.6% of patients and 6.3% of controls, which were not significant in the $\chi^2$ test. Pathological $\alpha_2$-MG values were measured in 17.9% and 27.5%, which were not significant in the $\chi^2$ test either.

**$\alpha_1$-AT Genotypes**

An equal number of Pi-MM genotypes was identified in patients and controls. The allele frequencies found in the control group are 0.9521 for Pi-M, 0.0342 for Pi-S, and 0.0137 for Pi-Z. The allele frequencies in the patients with sCAD were 0.9333 for Pi-M, 0.0267 for Pi-S, and 0.0400 for Pi-Z. There was an almost equal distribution of the Pi-MS genotype (Table 3). The overall distribution of $\alpha_1$-AT genotypes did not differ statistically between patients and controls. Introducing 1-AT deficiency as a covariate does not change this result. For Pi-ZZ, there was no significant difference between cases and controls. Introducing CRP as a covariate does not change this result.

### Discussion

Abnormal proteolytic activity and altered $\alpha_1$-AT genotypes have so far been described in patients with arterial aneurysms and fibromuscular dysplasia. A number of case reports demonstrated an association between $\alpha_1$-AT deficiency and arterial aneurysms. Protease-antiprotease imbalance was revealed by measurements of the elastase-$\alpha_1$-AT balance in tissue of aortic aneurysms and in the serum of patients with ruptured cerebral aneurysms. Genetic studies provided divergent results, some suspecting an association of $\alpha_1$-AT variants with arterial aneurysms, others questioning such an association.

### Table 2. $\alpha_1$-AT and $\alpha_2$-MG Serum Levels

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients: Median, Mean, and SD</th>
<th>Controls: Median, Mean, and SD</th>
<th>Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1$-AT, $\mu$mol/l</td>
<td>130.5</td>
<td>124.5</td>
<td></td>
</tr>
<tr>
<td>$\alpha_2$-MG, $\mu$mol/l</td>
<td>159.5</td>
<td>146.5</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. $\alpha_1$-AT Polymorphism**

<table>
<thead>
<tr>
<th>$\alpha_1$-AT Genotype</th>
<th>Patients</th>
<th>Controls</th>
<th>Fisher Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi-MM</td>
<td>66</td>
<td>66</td>
<td>P=0.839</td>
</tr>
<tr>
<td>Pi-MS</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Pi-MZ</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pi-ZZ</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4. Patients With $Z$ Alleles**

<table>
<thead>
<tr>
<th>$\alpha_1$-AT Genotype</th>
<th>$\alpha_1$-AT Levels, $\mu$mol/l</th>
<th>Age</th>
<th>Gender</th>
<th>Smoking</th>
<th>Dissected Vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi-MZ</td>
<td>80.0</td>
<td>54</td>
<td>male</td>
<td>no</td>
<td>Left ICA</td>
</tr>
<tr>
<td>Pi-MZ</td>
<td>86.9</td>
<td>43</td>
<td>female</td>
<td>no</td>
<td>Left ICA</td>
</tr>
<tr>
<td>Pi-MZ</td>
<td>72.6</td>
<td>38</td>
<td>female</td>
<td>no</td>
<td>Left and right ICA</td>
</tr>
<tr>
<td>Pi-MZ</td>
<td>74.2</td>
<td>37</td>
<td>female</td>
<td>no</td>
<td>Right ICA</td>
</tr>
<tr>
<td>Pi-ZZ</td>
<td>20.9</td>
<td>45</td>
<td>male</td>
<td>no</td>
<td>Left ICA</td>
</tr>
</tbody>
</table>

*A detailed description of the patient with Pi-ZZ is given elsewhere.*

For fibromuscular dysplasia, an association with $\alpha_1$-AT deficiency is also suspected.

For sCAD, 6 case reports and 1 case series suggest an etiologic role of $\alpha_1$-AT for spontaneous arterial dissections: dissection of the internal and external iliac arteries occurred in a 34-year-old man with Pi-SZ genotype, a dissecting hematoma of the left coronary trunk in an $\alpha_1$-AT-deficient 46-year-old woman, ICA dissections occurred in a 38-year-old woman and a 50-year-old man with Pi-MZ genotype, and sCAD with multiple aneurismal dilatations in a man with M,S genotype. Our group reported a 45-year-old male patient with Pi-ZZ genotype who had spontaneous ICA dissection with embolic middle cerebral arterial occlusion and was successfully treated by systemic thrombolysis. Recently, an increased rate of lowered $\alpha_1$-AT levels was observed in a series of 22 sCAD patients, whereas no association of $\alpha_1$-AT with sCAD was found in 35 patients (16 in the acute phase, 19 in the convalescent phase). The reports cited above raise the possibility that reduced levels of protease inhibitors, such as $\alpha_1$-AT and $\alpha_2$-MG, might be a risk factor for sCAD. However, patient numbers studied so far are much too small to generalize for the results, and underlying genetics were only analyzed in single cases. One very recent investigation of $\alpha_1$-AT deficiency alleles in 74 patients did not find a relationship between $\alpha_1$-AT alleles and sCAD; however, $\alpha_1$-AT levels and other protease inhibitors were not investigated.

This study investigates the protease inhibitor hypothesis, including a systematic genetic analysis in a relatively large number of patients, considering the low annual incidence of this disease. Because of this fact, a retrospective study design was chosen, accepting the limitation that only patients who were able and willing to give written informed consent and blood samples up to 10 years after sCAD could be included. A strength of this study is the well-matched control population originating from a large population-based study. The allele frequencies found in the present control cohort are within the range estimations of 0.9272 to 0.9708 for Pi-M, 0.0176 to 0.0564 for Pi-S, and 0.0074 to 0.0153 for Pi-Z for different European regions. Because the regional provenance plays an important role, it is advantageous that the present control cohort was recruited within the same region of Germany (Westfalia) than the patient group. Rare $\alpha_1$-AT deficiency alleles and null alleles genotypes occurring with frequencies like 1.1×10^{-4} for Pi*Mmalton, 2.5×10^{-5} for Pi*Mcobalt, or 1.410^{-4} for all null alleles combined were not included in the genetic analysis. However, these variants...
lead to a dramatic reduction of α₁-AT serum levels to 3% to 15% of their normal values or <1% for null alleles.42 That all α₁-AT serum levels <90 μmol were associated with either Pi-S or Pi-Z alleles makes it unlikely that we overlooked one of the rare variants. Acute phase reactions leading to artificially increased α₁-AT levels were avoided by testing patients >1 month after dissection. Nevertheless, the statistic power of the present sample is insufficient to test whether a combination of genetically determined protease inhibitor deficiency with acquired risk factors, such as smoking, recent infection, or reduced vitamin levels may increase the risk of sCAD.

In summary, this data does not support the hypothesis that protease inhibitor levels or α₁-AT genotypes play an important role in the etiology of sCAD. The frequency of Z alleles in the patient group was higher than in the control group and than in other cohorts from Europe; however, the difference remained nonsignificant. Surprisingly, all patients with Z alleles had ICA and not VA dissections. Differences in the pathobiology of VA and ICA dissections have so far not been described. However, our group has reported that VA dissections are more often preceded by minor trauma-like chiropractic manipulations than ICA dissections, pointing toward different causative mechanisms.45 Future research might therefore consider VA and ICA dissections separately.

Acknowledgments
Studies on sCAD at the University of Münster are supported by a grant from the Deutsche Schlaganfallhilfe and the Bundesministerium für Bildung und Forschung (Kompetenznetz Schlaganfall, 01GI 2909/3).

References


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Stroke. 2005;36:9-13; originally published online November 18, 2004; doi: 10.1161/01.STR.0000149631.52985.27
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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